

**IN THE CLAIMS:**

1. (Currently amended) A liquid pharmaceutical composition in the form of an aqueous solution comprising an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, a multiply charged inorganic anion, a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0, ~~and~~ said product being present in said solution in a sufficient amount to provide a therapeutically effective amount of said product when the solution ~~or a portion of said solution~~ is administered to a patient, said liquid composition being stable at room temperature.

2. (Original) The composition of claim 1 wherein said solution is an isotonic solution.

3. (Original) The composition of claim 1 wherein the anion is an anion of a multiple charged strong inorganic acid.

4. (Original) The composition of claim 3 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

5. (Original) The composition of claim 4 wherein the anion is a sulfate anion.

6. (Original) The composition of claim 1 wherein the pH is 5.8 to 6.7

Serial No. 09/853,731

Filed: May 11, 2001

7. (Original) The composition of claim 6 wherein the pH is 6.0 to 6.5
8. (Original) The composition of claim 7 wherein the pH is about 6.2.
9. (Original) The composition of claim 1 wherein the buffer is selected from the group consisting of a phosphate or arginine/H<sub>2</sub>SO<sub>4</sub>/Na<sub>2</sub>SO<sub>4</sub> buffers.
10. (Original) The composition of claim 9 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.
11. (Original) The composition of claim 1 wherein the product is a human erythropoietin.
12. (Currently amended) The composition of claim 11 wherein the ~~product is expressed~~ human erythropoietin is produced by endogenous gene activation.
13. (Original) The composition of claim 12 wherein the erythropoietin has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.
14. (Currently amended) The composition of claim ~~11~~ 13 wherein the amino acid sequence of the erythropoietin ~~product has the sequence of human erythropoietin~~ is modified by the addition of from 1 to 6 glycosylation sites.

15. (Currently amended) The composition of claim 14 wherein the sequence modification is selected from the group consisting of:

Asn<sup>10</sup>Thr<sup>32</sup>;  
Asn<sup>51</sup>Thr<sup>53</sup>;  
Asn<sup>57</sup>Thr<sup>59</sup>;  
Asn<sup>69</sup>;  
Asn<sup>69</sup>Thr<sup>71</sup>;  
Ser<sup>68</sup>Asn<sup>69</sup>Thr<sup>71</sup>;  
Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
Ser<sup>87</sup>Asn<sup>88</sup>Gly<sup>89</sup>Thr<sup>90</sup>;  
Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Thr<sup>92</sup>;  
Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Ala<sup>162</sup>;  
Asn<sup>69</sup>Thr<sup>71</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;  
Ser<sup>87</sup>Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;  
Asn<sup>136</sup>Thr<sup>138</sup>;  
Asn<sup>138</sup>Thr<sup>140</sup>;  
Thr<sup>125</sup>; and  
Pro<sup>124</sup>Thr<sup>125</sup>.

16. (Currently amended) The composition of claim ~~113~~, wherein the erythropoietin ~~said glycoprotein product~~ has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

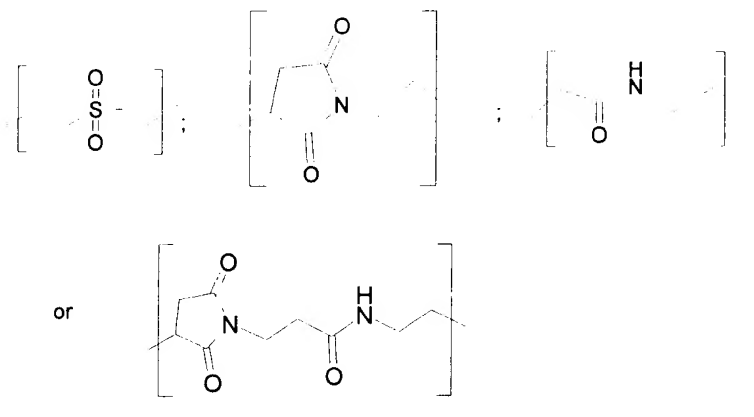
17. (Original) The composition of claim 16, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin sequence with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

18. (Cancelled)

19. (Original) The composition of claim 1, wherein the glycoprotein product is a pegylated erythropoietin.

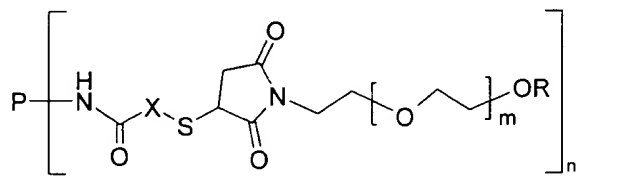
Claims 20-22 (Cancelled)

23. (Currently amended) A liquid pharmaceutical composition in the form of an aqueous solution comprising a pegylated ~~The composition of claim 19, wherein the pegylated erythropoietin is a conjugate of an erythropoietin glycoprotein conjugate, said glycoprotein having at least one free amino group and, said glycoprotein having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 of human erythropoietin~~ or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein *via* a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups; X is -(CH<sub>2</sub>)<sub>k</sub>- or -CH<sub>2</sub>(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>k</sub>-; k is from 1 to 10; Y is selected from



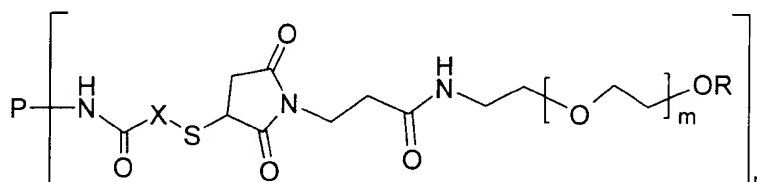
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons; and wherein said conjugate having a the molecular weight of the pegylated erythropoietin glycoprotein is from about 51 kilodaltons to about 175 kilodaltons; said liquid composition being stable at room temperature.

24. (Currently amended) The composition of claim 23 wherein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is  $-(\text{CH}_2)_k-$  or  $-\text{CH}_2(\text{O}-\text{CH}_2-\text{CH}_2)_k-$ ; and P is the residue of the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is as above from 1-10.

25. (Currently amended) The composition of claim 23 wherein the ~~wherein said~~ conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is  $-(\text{CH}_2)_k-$  or  $-\text{CH}_2(\text{O}-\text{CH}_2-\text{CH}_2)_k-$ ; and P is ~~the residue of~~ the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is ~~as above~~ from 1-10.

26. (Currently amended) A liquid pharmaceutical composition in the form of an aqueous solution comprising from 10  $\mu\text{g}$  to 10,000  $\mu\text{g}$  per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, from 10 to 200 mmol per liter of said solution of a multiply charged inorganic anion and from 10 to 50 mmol per liter of said solution of a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0; said liquid pharmaceutical composition being stable at room temperature.

27. (Original) The composition of claim 26 wherein said solution is an isotonic solution.

Serial No. 09/853,731

Filed: May 11, 2001

28. (Original) The composition of claim 26 wherein the anion is an anion of a multiple charged strong inorganic acid.

29. (Original) The composition of claim 28 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

30. (Original) The composition of claim 29 wherein the anion is a sulfate anion.

31. (Original) The composition of claim 30 wherein the pH is 5.8 to 6.7

32. (Original) The composition of claim 30 wherein the pH is 6.0 to 6.5

33. (Original) The composition of claim 31 wherein the pH is about 6.2.

34. (Original) The composition of claim 26 wherein the buffer is selected from the group consisting of phosphate or arginine/ $\text{H}_2\text{SO}_4$ / $\text{Na}_2\text{SO}_4$  buffers.

35. (Original) The composition of claim 34 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.

36. (Original) The composition of claim 26 wherein the product is a human erythropoietin.

37. (Original) The composition of claim 36 wherein the product is expressed by endogenous gene activation.

38. (Currently amended) The composition of claim 37 wherein the erythropoietin has the amino acid sequence ~~of~~ SEQ ID NO:1 or SEQ ID NO:2.

39. (Currently amended) The composition of claim 26 wherein said the erythropoietin glycoprotein product has the sequence SEQ ID NO: 1 or SEQ ID NO: 2 ~~of human erythropoietin~~ that is modified by the addition of from 1 to 6 glycosylation sites.

40. (Currently amended) The composition of claim 39 wherein the sequence of modification is selected from the group consisting of:

Asn<sup>30</sup>Thr<sup>32</sup>;

Asn<sup>51</sup>Thr<sup>53</sup>;

Asn<sup>57</sup>Thr<sup>59</sup>;

Asn<sup>60</sup>;

Asn<sup>60</sup>Thr<sup>71</sup>;

Ser<sup>68</sup>Asn<sup>60</sup>Thr<sup>71</sup>;

Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Gly<sup>89</sup>Thr<sup>90</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Thr<sup>92</sup>;

Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Ala<sup>162</sup>;

Asn<sup>60</sup>Thr<sup>71</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;

Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;

Ser<sup>87</sup>Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;



~~Asn<sup>136</sup>Thr<sup>138</sup>;~~

~~Asn<sup>138</sup>Thr<sup>140</sup>;~~

~~Thr<sup>125</sup>; and~~

~~Pro<sup>124</sup>Thr<sup>125</sup>.~~

41. (Currently amended) The composition of claim 26 wherein said erythropoietin glycoprotein product has the sequence SEQ ID NO: 1 or SEQ ID NO: 2 ~~that is of human erythropoietin~~ modified by a rearrangement of at least one glycosylation site.

42. (Original) The composition of claim 41, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

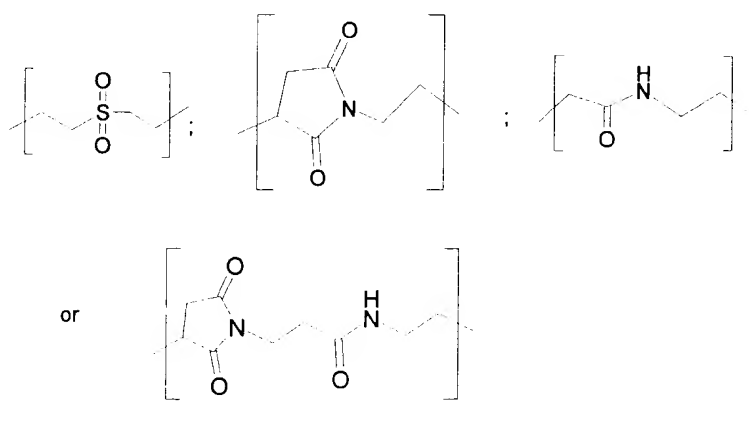
43. (Cancelled)

44. (Currently amended) The composition of claim 26, wherein said glycoprotein product is a pegylated erythropoietin glycoprotein product.

Claims 45-47 (Cancelled)

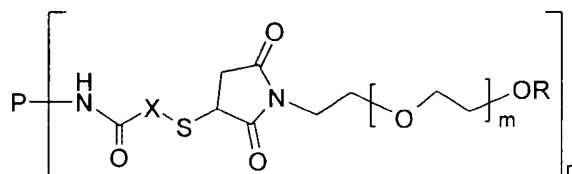
48. (Currently amended) The composition of claim 44, wherein the pegylated erythropoietin is a conjugate of an erythropoietin glycoprotein having at least one free amino group, said glycoprotein having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 ~~of human erythropoietin~~ or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene

glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein via a linker of the formula  $-C(O)-X-S-Y-$  with the  $C(O)$  of the linker forming an amide bond with one of said amino groups, X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ ; and k is from 1 to 10; Y is selected from



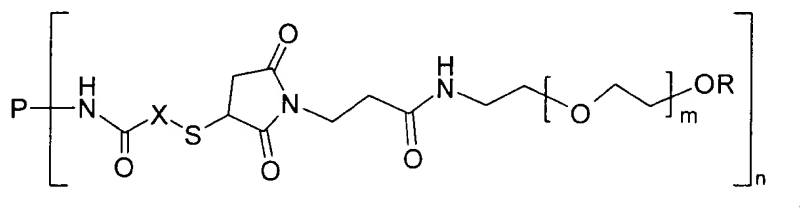
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate being from about 51 kilodaltons to about 175 kilodaltons.

49. (Currently amended) The composition of claim 48 wherein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , and P is ~~the residue of the erythropoietin glycoprotein without~~ minus the amino group or groups which form an amide linkage with X and k is from 1 to 10, as above.

50. (Currently amended) The composition of claim 49 ~~wherein~~ wherein said conjugate has the formula:



n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , and P is ~~the residue of the erythropoietin glycoprotein without~~ minus the amino group or groups which form an amide linkage; and k is from 1 to 10, as above.

51. (Currently amended) The composition of claim 26 wherein said solution contains 10  $\mu\text{g}$  to 10000  $\mu\text{g}$  erythropoietin protein per ml of solution, from 10 to 200 mmol/liter of solution of ~~the~~ a sulfate as the multiply charged inorganic anionsulfate, and 10 to 50 mmol/liter of solution of a phosphate as the pharmaceutically acceptable buffer, phosphate, said solution having and a pH of from about 6.0 to about 6.5.

52. (Currently amended) The composition of claim 51 further comprising up to 20 mM per liter of methionine, and 1 - 5 % of a polyol (w/v).

53. (Currently amended) The composition of claim 52 comprising 10  $\mu$ g to 10000  $\mu$ g erythropoietin protein per ml of solution, 40 mmol/liter of solution of the sulfate, 10 mmol/liter of said solution of the phosphate, ~~3% mannitol (w/v)~~, 10 mM methionine, ~~and said composition having a pH of about 6.2, and wherein the polyol is mannitol which is present in the solution at 3% (w/v).~~

54. (Currently amended) The composition of claim 26 wherein ~~the said solution contains 10  $\mu$ g to 10000  $\mu$ g erythropoietin protein per ml of solution, the inorganic anion is NaCl which is present at 10 to 100 mmol/liter of solution, of NaCl, the buffer is 10 to 50 mmol/liter of solution of phosphate which is present at 10 to 50 mmol/liter of solution, said solution having at a pH of from about 6.0 to about 7.0.~~

55. (Currently amended) The composition of claim 54 ~~wherein said solution comprises 10  $\mu$ g to 10000  $\mu$ g erythropoietin protein per ml of solution, wherein the NaCl is present at 100 mmol/liter of solution, of NaCl, 10 mM methionine, the phosphate is present at and 10 mmol/l, phosphate, pH 7.0~~ said solution further comprising 10 mM methionine and having a pH of about 7.0.

56. (Currently amended) The composition of claim 26 wherein said solution comprises 10  $\mu$ g to 10000  $\mu$ g erythropoietin protein per ml of said solution, the anion is sodium sulfate which is present at 10 to 100 mmol/liter of solution, the buffer is arginine which is present at 10 to 50 mmol/liter of ~~said solution of arginine~~, said solution having a pH of about 6 to about pH 6.5, ~~10 to 100 mmol/liter of solution of sodium sulfate.~~

57. (Currently amended) The composition of claim 56 wherein said solution comprises 10 µg to 10000 µg erythropoietin protein per ml, 40 mmol/liter of solution of arginine, pH 6.2, 30 mmol/l sodium sulfate, said solution further comprising 3 % mannitol, 10 mM methionine, and 0.01% pluronic F68, and having a pH of about 6.2.

58. (Cancelled)

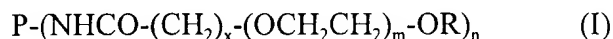
59. (Currently amended) The composition of claim 26 wherein the amount of erythropoietin is selected from 50, 100, 400, 800 or 2,500 µg/ml of solution.

60. (Currently amended) The composition of claim 59 comprising 10 mM sodium phosphate, 40 mM sodium sulfate, 3% mannitol, 10 mM methionine, 0.01% pluronic F68, and a having a pH of about 6.2.

61. (Currently amended) The composition of claim 60 comprising 40 mM arginine, 30 mM sodium sulfate, 3% mannitol, 10 mM methionine, 0.01% pluronic F68, and having a pH of about 6.2.

Claims 62-66 (Cancelled)

67. (New) A liquid pharmaceutical composition in the form of an aqueous solution comprising a therapeutically effective amount of a pegylated erythropoietin glycoprotein product of formula



wherein

P is an erythropoietin glycoprotein having the sequence SEQ ID NO: 1, SEQ ID NO: 2, or either of these sequences modified by the addition of from 1 to 6 glycosylation sites or by rearrangement of at least one glycosylation site, minus the n amino group of said glycoprotein,

R is lower alkyl,

x is 2 or 3,

m is from about 450 to about 900;

n is from 1 to 3; and

wherein the values of n and m are such that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons; and

a multiply charged inorganic anion and a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount such that the pH of the solution is from about 5.5 to about 7.0.

68. (New) The composition of claim 67 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

69. (New) The liquid pharmaceutical composition of claim 67 wherein the pegylated erythropoietin glycoprotein product is present in an amount of from about 10 µg to about 10,000 µg per ml of said liquid composition, the multiply charged inorganic anion is present in an amount of from 10 to 200 mmol per liter of said liquid composition, and the pharmaceutically

acceptable buffer is present in an amount of from about 10 to about 50 mmol per liter of said liquid composition, said anion and said buffer being present in said liquid composition in an amount to provide a pH of from about 5.5 to about 7.0.

70. (New) The composition of claim 69 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

71. (New) The liquid pharmaceutical composition of claim 68 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 100.0 µg/mL of solution, the multiply charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg, and wherein the pH of the solution is about  $6.2 \pm 0.2$ .

72. (New) The liquid pharmaceutical composition of claim 71 further comprising methionine in an amount of about 1.49 mg, mannitol in an amount of about 30.0 mg and poloxamers type 188 in an amount of 0.1 mg.

73. (New) The liquid pharmaceutical composition of claim 68 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 400 µg/mL of solution, the multiply charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg, and wherein the pH of the solution is about  $6.2 \pm 0.2$ .

74. (New) The liquid pharmaceutical composition of claim 73 further comprising methionine in an amount of about 1.49 mg, mannitol in an amount of about 30.0 mg and poloxamers type 188 in an amount of 0.1 mg.

75. (New) The liquid pharmaceutical composition of claim 68 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 800.0  $\mu\text{g/mL}$  of solution, the multiply charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg, and wherein the pH of the solution is about  $6.2 \pm 0.2$ .

76. (New) The liquid pharmaceutical composition of claim 75 further comprising methionine in an amount of about 1.49 mg, mannitol in an amount of about 30.0 mg and poloxamers type 188 in an amount of 0.1 mg.

77. (New) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500  $\mu\text{g/mL}$ , the buffer is sodium or potassium phosphate which is present in an amount of about 10 mM, the anion is NaCl which is present in an amount of about 100 mM, said composition having a pH of about 7.0.

78. (New) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500  $\mu\text{g/mL}$ , the buffer is sodium phosphate which is present at about 10 mM, the anion is sodium sulfate which is present at about 120 mM, said composition having a pH of about 6.2.



79. (New) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500  $\mu\text{g/ml}$ , the buffer is sodium phosphate which is present at about 10 mM, the anion is sodium sulfate which is present at about 40 mM, said composition further comprising 3% mannitol and having a pH of about 6.2.

80. (New) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500  $\mu\text{g/ml}$ , the buffer is sodium phosphate which is present at about 10 mM, the anion is sodium sulfate which is present at about 40 mM, said composition further comprising 3% mannitol and 10 mM methionine and having a pH of about 6.2.

81. (New) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500  $\mu\text{g/ml}$ , the buffer is arginine which is present at about 40 mM, the anion is sodium sulfate which is present at about 30 mM, said composition further comprising 3% mannitol and having a pH of about 6.2.

82. (New) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500  $\mu\text{g/ml}$ , the buffer is arginine which is present in an amount of about 40 mM, the anion is sodium sulfate which is present in an amount of about 30 mM, said composition further comprising 3% mannitol and 10 mM methionine and having a pH of about 6.2.